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THE SPERMATOGENESIS OF XIPHIDIUM FASCIATUM, . . *C. E. McClung.*

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THE SPERMATOGENESIS OF XIPHIDIUM FASCIATUM.

BY C. E. McCLUNG.

(Contribution from the Zoölogical Laboratory, No. 178.)

Plate XV.

IN 1898 I described, very briefly, certain stages in the spermatogenesis of *Xiphidium*. This was merely a preliminary paper, and contained nothing beyond an account of certain changes undergone by the accessory chromosome. At the time it was my intention to complete a study of the maturation phenomena in this locustid, but very shortly afterward I encountered in the *Acrididæ* material much better suited to the purpose of my studies and I put the *Xiphidium* preparations aside. Later, when I again turned to the locustids, I found so much larger and clearer cells in *Anabrus* and *Orchesticus* than in *Xiphidium* that it seemed undesirable to use the earlier material except in a comparative way.

That there was a real similarity in the essential features of the process throughout the family I was led to believe by the study of numerous species, and to indicate this most clearly I entitled the paper "The Spermatocyte Divisions of the *Locustidæ*." Notwithstanding this and several specific statements that the descriptions of processes were to apply to the family, including of course *Xiphidium*, with but few exceptions authors and reviewers have based their understanding of my views upon the earlier preliminary paper.

In order, therefore, to avoid further misunderstanding upon the subject, I have recently undertaken a restudy of my *Xiphidium* material, for the purpose of publishing a more complete account of the spermatogenesis of this species. With a broader experience and a better material equipment than I possessed

when I published my first cytological paper, in 1898, I am able to interpret the somewhat obscure phenomena of maturation in this species more accurately than I could then. So far as essentials are concerned, however, I find nothing to change from the accounts in the previous papers, and I shall, for that reason, not go into any great detail in my descriptions. Since other investigators, utilizing similar material, have published accounts that differ from my own in some particulars, I shall, upon these points, speak more extensively. For the sake of completeness I purpose to consider the different generations of cells and to mention the phenomena that each exhibits, particularly those relating to the accessory chromosome.

SPERMATOGONIA.

A determination of the changes experienced by the elements of this generation of cells is very difficult on account of the small size of the cells, the number of chromosomes, and the tendency of the chromosomes to fuse together under the action of the fixing agents. It may be stated with reasonable certainty, however, that the number of chromosomes is the typical one of the family. Of these thirty-three elements the accessory chromosome is by far the most prominent in all the stages of mitosis. The insertion of its fiber is median, and the halves of the dividing chromosome go to the poles of the spindle as U-shaped loops. While the limbs of the U are generally parallel, they are sometimes widely separated, thus forming a V-shaped figure. Because of their length, these parts of the accessory chromosome remain for some time with their ends in the region of the equatorial plate, but they are gradually withdrawn into the general mass of chromatin at the ends of the spindle. (Figs. 1, 2, 3, 4, plate XV.)

The ordinary chromosomes show some little diversity in size, but they do not present favorable conditions for the study of chromosome structure and behavior. Because of their number, and the restrictions placed upon them by the size of the cell, they form an almost solid mass of chromatin in the equatorial plate during the metaphase. Favorable preparations show, however, that the individual elements are distinct and united together only by linin threads. In most cells, on the contrary, the fixation has caused the confluence of all the chromosomes into a plate with occasional apertures such as I described in my former paper ('99, p. 188).

FIRST SPERMATOCYTES.

During the prophase of the first spermatocytes there occur changes in the chromatin elements that make it possible to determine something of their structure and final disposition. With regard to the accessory chromosome I have not much to add to my former descriptions ('99, '02). A spireme stage, such as I figured for *Orchesticus* ('02, fig. 5), appears, but not so clearly as in other genera of the family. This would seem to be a common condition, for it has been recognized in *Locusta* by Otte ('06, p. 531), who apparently was unaware of my earlier account.

Because of the mistaken interpretation by this author of the changes experienced by the accessory chromosome it would seem advisable to present the history of this element somewhat more in detail than I have before done. The long, slender, much coiled thread of the spireme gradually shortens, thickens, and uncoils, the result being to produce, generally, a thread bent upon itself at or near the middle (fig. 5). This double thread may lie straight, but more commonly is convoluted with the free ends in approximation. There is, however, no fixed type form, and almost any shape that a flexible thread may take is represented. As the thread shortens and thickens still further the irregularities of form decrease in number. There is still manifest a tendency for the thread to be bent, usually near the middle, but not infrequently it is straight. Finally, as it approaches the metaphase, it takes on the boomerang form described in my earlier paper ('99, p. 190).

The longitudinal cleavage of the thread is not at any time very apparent, but indications of it are not lacking. The point of interest at this stage is that before the metaphase is reached the rod has almost invariably become almost straight, and never shows the U-shape of the early prophase. It was the failure of Otte to notice this point that led him into error with regard to the division of the accessory chromosome.

The ordinary chromosomes of the prophase show the typical rings, rods and crosses that have now so often been described in insect spermatocytes. There is every reason to believe that they conform to the type of tetrad that is found in other members of the order. No unusual phenomena were observed.

During the metaphase of the first spermatocyte a clearer

conception of the numerical and spatial relations of the chromosomes may be observed than was possible in the earlier stages. At this time it is very apparent from inspections of polar views that the number of ordinary chromosomes is sixteen. There exists also some variation in the sizes of the chromosomes, and two of larger dimensions may always be recognized on the periphery of the equatorial plate, and usually together. The outlines of the chromosomes, in polar view, are approximately circular and no rings are visible. A very compact equatorial plate is formed with little space between the chromosomes. No regular arrangement of the elements was noticeable, except that the larger ones are on the periphery. Occasionally the accessory chromosome, in addition to the sixteen ordinary ones, may be seen in polar view (figs. 6, 7, 8, 9).

A lateral view of the first spermatocyte metaphase shows a series of dumb-bell-shaped chromosomes with now and then a ring-shaped element. There may also be observed lobate chromosomes like a letter E with the middle bar absent. In a large majority of cases the accessory chromosome has already passed to one pole of the spindle without division. The position described in my former paper ('99, p. 190) is unusual, and there can be no question that the accessory chromosome is not divided in the first spermatocyte mitosis (figs. 10, 11). It is impossible to ascertain the character of the chromosome division in this generation of cells, for the elements are almost all of one shape, and offer no means for the determination of planes or axes. In the case of the ring- and E-shaped chromosomes, because of their position, I am inclined to believe that there occurs a segregation division. I hope to present in another paper the evidence for my belief that rings of this character are divided transversely while those that lie in the plane of the equatorial plate, such as I have described for other species of the Orthoptera, are longitudinally cleft. The proof in support of this view seems to me very conclusive, and offers the means for reducing much of the discrepancy that exists in the conclusions of different observers. *Xiphidium*, however, does not afford material suitable for the determination of such points, and I have made no effort to utilize it in this way.

Telophases of the first spermatocyte show the very large accessory chromosome in one daughter-cell and absent from the other. There is evident at this period a marked separation of

the chromatids of this element, usually more prominently at the ends, while at the center they remain in contact. The result is to produce a structure that looks like two U's with their rounded ends in contact. A careful study shows that this is merely an apparent condition, for other cells have the accessory chromosome with the chromatids parallel (fig. 12). The ordinary chromosomes become very loose and granular, but their outlines may still be seen indistinctly.

SECOND SPERMATOCYTES.

The prophase of the second spermatocyte is very brief, apparently, and soon the chromosomes have taken on their homogeneous condition, and have arranged themselves in the equatorial plate. Only rarely, however, do they show the sharp outlines found in chromosomes of the preceding cell generation, but more frequently tend to coalesce somewhat. A polar view of the metaphase shows a compact plate of chromosomes with the large ones on the periphery. In those cells possessing the accessory chromosome there is added this element to the peripheral series. In form the accessory chromosome is V- or U-shaped, with the apex directed toward the center of the spindle (figs. 13, 14, 15). When viewed from the side the V- or U-shaped element is discovered to be double. The ordinary chromosomes are again dumb-bell-shaped (fig. 16).

An inspection of an early anaphase of the second spermatocyte shows that the mantle fibers attach to the center of the chromatid in the accessory chromosome, and division commences therefore at this point. In mid-anaphase the accessory chromosome has accordingly the form of two V's with their apices directed away from each other and toward the poles of the spindle. Still later the separated V's are found with the other chromosomes at the poles of the spindle. Even in the telophase they remain in this form and position after the other chromosomes have fused together in a mass. The plane of separation is unquestionably the longitudinal cleft, visible most prominently in the telophase of the first spermatocyte. In those second spermatocytes from which the accessory chromosome is absent, the chromatin elements divide almost synchronously and pass to the pole of the spindle in a mass. The presence of the accessory chromosome, which divides later than the other chromosomes, tends to retard division on its side of

the plate, and the daughter groups are therefore inclined to each other instead of being parallel. No other peculiarities in the division of the ordinary chromosomes presented themselves. (Figs. 17, 18, 19, 20.)

GENERAL CONSIDERATIONS.

The points of interest in the spermatogenesis of *Xiphidium* relate to the accessory chromosome, and I shall therefore first briefly summarize its history and then speak of the accounts of other investigators.

It is to be noted that the accessory chromosome is a single, very large spermatogonial chromosome that enters the prophase of the first spermatocyte without undergoing any extensive separation of its chromomeres. While it forms a thread, this is always denser and more compact than the general spireme. The result is that the element always stains strongly, after the manner of metaphase chromosomes. It is thus made very conspicuous in its position on the periphery of the nucleus. It passes through a variety of forms, but finally enters the metaphase of the first spermatocyte as a bent rod, and in this form passes *undivided* to one pole of the spindle. The result is to produce two types of second spermatocytes.

In the metaphase of the second spermatocyte the accessory chromosome takes its place in the equatorial plate and is there divided along the plane of its early longitudinal cleavage—an equation division. From each first spermatocyte there are accordingly produced four spermatids, two of which possess an accessory chromosome while the other two are without it. These results are in accordance with my more extensive work ('02) upon other members of the same family, and vary at points from the preliminary account upon *Xiphidium*.

The only other investigators that have concerned themselves with the spermatogenesis of the locustids are dé Sinety ('01), Stevens ('05), and Otte ('06). The work of Sabatier ('90) dealt only with the transformation stages, and so does not concern the present discussion. The results of dé Sinety and Stevens in the main agree with mine; those of Otte are radically different in essentials. I wish here briefly to note the work of the latter author upon the accessory chromosome only. I hope to consider the maturation divisions of insects in detail in a subsequent paper.

I consider the work of Otte entirely erroneous so far as it relates to the method of division of the accessory chromosome in the second spermatocyte mitosis, for it is widely at variance with what I have found in the large number of species of the Orthoptera that I have examined. The figures that he presents in his preliminary paper ('06) are not at all convincing, and I am fully persuaded that a more thorough study will convince him that his identification of the longitudinal split of the accessory chromosome in the second spermatocyte metaphase with the space between the bent halves of the rod in the prophase of the first spermatocyte is a mistake. That Otte's interpretation could not apply to *Xiphidium* is evident from an inspection of the second spermatocyte during the division of the accessory chromosome (fig. 17). The element here is in the shape of a V with the fibers attached at the angle. It is in this form that Otte conceives the break across the rod to occur, but instead the whole bent structure is split along its length into duplicate V-shaped halves. There is no possibility of mistaking the longitudinal split for a space between the halves of a bent rod. In those forms where the fibers attach at the ends of the chromosomes there would be more of a possibility of such confusion, but even there a sufficiently complete series of stages will make clear the presence of the longitudinal cleavage and its identity with the plane of separation in the second spermatocyte metaphase.

It is unnecessary to point out that Otte's account does not agree with the great majority of observations upon other forms, and that, on the theoretical side, it attacks the hypothesis of the individuality of the chromosomes, unless indeed he would assume that the accessory chromosome is bivalent. It is very probable that he does not make this assumption, for all the other chromosomes are described as being transversely divided. So far as I know, the only writer who regards the accessory chromosome as bivalent in the Orthoptera is Montgomery, who made a superficial study of this structure in *Syrbula*, an acridian. That he is in error on this point is shown by the work of Robertson (in publication) upon the same genus.

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EXPLANATION OF PLATE XV.

The original *camera lucida* drawings are at a magnification of 3000 diameters, and appear here at 2000. The optical equipment with which the preparations were studied consists of an apochromatic objective of 2 mm. focus and 1.40 N. A., and compensating oculars. The light of the Welsbach mantle was delivered to the objective through a Watson oil-immersion condenser of 1.30 N. A.

FIG. 1.—Telophase of secondary spermatogonium, showing the ends of the divided accessory chromosome almost in contact.

FIG. 2.—Telophase of similar cell, showing the accessory chromosome *en face*.

FIG. 3.—Same stage as figure 2.

FIG. 4.—Same as figure 2, but with the halves of the accessory chromosome more separated.

FIG. 5.—Various forms of the accessory chromosome in the prophase of the first spermatocyte. Those represented at *a*, *b*, *c*, were fixed in Flemming's fluid and stained in iron-hæmatoxylin; the remaining ones were fixed in Hermann's fluid and stained with safranin-gentian violet. The latter combination causes the elements to appear larger than the former. Variations in the form of the accessory chromosome, at about the same stage, are shown at *d*, *e*, *f*, *g*, *h*. The elements shown at *i*, *j*, *k*, *l*, *m*, *n*, are of later stage. Chromosomes *m* and *n* are from the same cyst, the former being a lateral view, the latter *en face*.

FIG. 6.—Polar view of a metaphase of the first spermatocyte, showing the sixteen ordinary chromosomes and the accessory chromosome beneath.

FIG. 7.—A slightly later stage than that represented in figure 6. No accessory chromosome shown.

FIG. 8.—Polar view of the first spermatocyte metaphase, showing the sixteen ordinary chromosomes.

FIG. 9.—Similar to figure 8.

FIG. 10.—Lateral view of a first spermatocyte metaphase, showing a few of the chromosomes. The one in the form of a ring lies in the plane of the spindle axis.

FIG. 11.—Same stage as represented in figure 10, but showing the accessory chromosome at one pole of the spindle.

FIG. 12.—Telophase of the first spermatocyte, showing the accessory chromosome in only one daughter-cell. 12*a* and 12*b* are views of the accessory chromosome in the same stage but with different degrees of chromatid separation.

FIG. 13.—Polar view of second spermatocyte metaphase in which the accessory chromosome is present.

FIG. 14.—Similar to the cell shown in figure 13.

FIG. 15.—Polar view of second spermatocyte without an accessory chromosome.

FIG. 16.—Lateral view of second spermatocyte with an accessory chromosome.

FIG. 17.—Mid-anaphase of second spermatocyte, showing separation of halves of the accessory chromosome.

FIG. 18.—Similar to figure 17.

FIG. 19.—Telophase of second spermatocyte, showing presence of the accessory chromosome in daughter-cells.

FIG. 20.—Telophase of second spermatocyte without the accessory chromosome.

FIG. 21.—Somatic cell in anaphase showing division of the accessory chromosome.

1
2
a
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c
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g
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k
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10
11
21
12
12a
12b
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